Maternal Serum Screening for Neural Tube Defects and Fetal Chromosome Abnormalities

NANCY C. ROSE, MD, and MICHAEL T. MENNUTI, MD, Philadelphia, Pennsylvania

Second-trimester maternal serum screening is a noninvasive means of identifying pregnant women at an increased risk for various conditions including a fetus with open spina bifida, fetal Down syndrome, trisomy 18, multiple gestation, and adverse pregnancy outcome. Combinations of several different markers are available for screening. These include α-fetoprotein, human chorionic gonadotropin, and unconjugated estriol. In this review, we discuss the benefits and limitations of the screening tests and the suggested protocols for the care of patients.

Maternal serum screening is the earliest noninvasive biochemical method of obtaining information about a fetus. The object of a medical screening test is to identify among a healthy population a small group of patients that is at a sufficiently increased risk of having a fetus with a disorder to be offered a specific diagnostic test. During pregnancy, biochemical screening markers are used to select the women who may be offered ultrasound examinations, amniocentesis, or other obstetrical intervention. The use of these prenatal screening tests has expanded from identifying fetuses at risk for neural tube defects to those with fetal chromosome abnormalities, as well as women at risk for third-trimester obstetrical complications. Maternal serum screening programs have the potential to decrease fetal morbidity and mortality by providing access to earlier diagnosis, by enabling families to make more informed reproductive decisions, and by designing appropriate delivery strategies. Combinations of three different markers are measured in maternal serum screening programs: α-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol.

Maternal Serum α-Fetoprotein Testing

Neural Tube Defects

Maternal serum AFP, the most commonly measured maternal serum marker, is ideal for fetal evaluation because under normal conditions it is produced solely by the fetus. Both high and low AFP values may be predictive of a birth defect or adverse pregnancy outcome. The association between the AFP value and fetal abnormalities was first recognized in 1972, when Brock and Sutcliffe measured AFP levels in the amniotic fluid of 31 pregnancies with anencephaly and 6 with spina bifida, hydrocephaly, or both.[1] All of the cases of anencephaly and most of the

spina bifida cases before 30 weeks' gestation were associated with amniotic fluid AFP levels that were markedly elevated. The basis for this observation is that when the fetus has an "open," or non–skin-covered defect, AFP leaks across the exposed capillaries from the fetal circulation into the amniotic fluid. The α-fetoprotein is also transferred across the amnion and the placenta into the maternal circulation. In 1974 Wald and co-workers did a case-controlled study comparing maternal serum AFP levels in 7 women carrying fetuses with open neural tube defects with 14 control women matched for maternal age, parity, and gestational age.[2] Maternal serum AFP values were significantly higher in the affected pregnancies than in the control population. This observation was validated by the United Kingdom Collaborative Study in 1977,[3] which demonstrated the usefulness of maternal serum AFP values in prospective screening tests for open neural tube defects.

Neural tube defects are the second most common serious fetal malformation in the United States, surpassed only by congenital heart defects. They are a heterogeneous group of disorders resulting from the failure of normal neural tube closure between the third and fourth week of embryologic development. The cranial end of the neural tube eventually becomes the forebrain, midbrain, and hindbrain, and a failure of closure results in acrania or anencephaly. The caudal end of the neural tube becomes the spinal cord, and a failure of closure results in spina bifida. A third type of neural tube defect, the encephalocele, is defined as cystic extensions of the brain through an overlying scalp and skin defect. Encephaloceles are much less common than spina bifida or anencephaly.

In the United States, the incidence of neural tube de-

ABBREVIATIONS USED IN TEXT

AFP = α-fetoprotein
hCG = human chorionic gonadotropin
MoM = multiple of the median

---

fects is 1 to 2 per 1,000 births. Their etiologic heterogeneity was noted by Holmes and associates, who reported that of 106 live-born or stillborn infants with a neural tube defect, about 12% had identifiable causes.[4] The various causes of these defects are listed in Table 1. Most (85%) neural tube defects are due to multifactorial inheritance—that is, a genetic predisposition due to an interplay between several genes and environmental factors—but 90% to 95% occur in couples without a family history of these defects.

At the most commonly applied cutoff levels, maternal serum AFP screening detects about 85% of cases of open fetal neural tube defects, consisting of 80% of fetuses with open spina bifida and 90% of those with anencephaly. The combination of maternal AFP screening and ultrasonography will detect more than 99% of cases of anencephaly. In contrast, most encephaloceles are skin covered[5(p191)] and are therefore less likely to be detected by maternal AFP screening or amniocentesis, but are more often diagnosed by ultrasonogram. Although maternal serum AFP screening was designed to detect neural tube defects, the use of this testing has been expanded to include many other abnormalities, including ventral wall defects, congenital nephrosis, and fetal death (Table 2).

Reporting Maternal Serum α-Fetoprotein Levels

The object of a screening test is to maximize detection at an acceptable false-positive rate. These biochemical markers are typically reported as a multiple of the median (MoM). This statistical convention was introduced by the First UK Collaborative Study on maternal serum AFP as a method for participating laboratories to compare individual test results.[3] The multiple of the median is a reflection of a patient's result compared with the laboratory's median value and is not influenced by outlying values. Every laboratory ideally develops its own reference data, with a median maternal serum AFP value from unaffected pregnancies calculated for each week of gestation. For AFP, the absolute value of a pregnant woman's AFP level is modified by other factors that affect the result. These include an inverse relationship with maternal weight, higher AFP levels in African Americans than in whites, and lower levels in women with insulin-dependent diabetes mellitus. The adjusted result is expressed as a multiple of the median by dividing the maternal serum AFP concentration by the median value for that week of gestation. A log gaussian distribution of maternal serum AFP levels is shown in Figure 1. The median maternal AFP value for each week of gestation is designated as 1.0 MoM. Most screening programs establish a cutoff of 2.0 or 2.5 times the median value (2.0 to 2.5 MoM) when compared with normal controls at the same week of ges-


TABLE 1.—Causes of Neural Tube Defects

Multifactorial inheritance
Single-gene (autosomal recessive) disorders
    Meckel's syndrome (most common)
    Robert's syndrome
    Jarcho-Levin syndrome
    Median facial cleft syndrome
    HARDE (Walker-Warburg) syndrome
    Oculoauriculovertebral dysplasia (Goldenhar's syndrome)
Chromosomal aneuploidy
    Trisomy 18
    Trisomy 13
    Trisomy 21
    Triploidy
    Unbalanced translocations, markers, ring chromosomes
Teratogenic agents
    Valproic acid
    Carbamazepine
    Aminopterin
    Thalidomide
Amniotic band sequence
Cloacal extrophy
Sacrococcygeal teratoma
Maternal insulin-dependent diabetes mellitus

HARDE = hydrocephalus, agyria, retinal dysplasia, and encephalocele

---

TABLE 2.—Abnormalities Identified by α-Fetoprotein Screening

Ventral wall defects
    Omphalocele
    Gastroschisis
Triploidy
Trisomies 18, 13, and 21
Unbalanced translocations
Amniotic band sequence
Pentalogy of Cantrell—omphalocele, lower sternal defect, deficiency of diaphragmatic pericardium, intracardiac abnormality, anterior diaphragmatic defect
Renal agenesis
Fetal demise
Multiple gestation
Congenital nephrosis (Finnish type)
Sacrococcygeal teratoma
Dermatologic disorders
    Epidermolysis bullosa
    Congenital ichthyosiform erythroderma
Chorioangioma
Risk of poor perinatal outcome
Maternal hepatoma
Maternal ovarian teratoma

---

tation. Each cutoff point is a balance between the detection rate and the false-positive rate: the higher the screening cutoff value, the lower the false-positive rate but the lower the detection rate. For example, at a 2.0-MoM cutoff, the false-positive rate is about 4%, whereas a 2.5 MoM cutoff has about a 2% false-positive rate. Selecting an appropriate cutoff level is also influenced by the prevalence of the disorder in the population to be screened. The most common causes of false-positive and false-negative maternal AFP results are listed in Table 3.
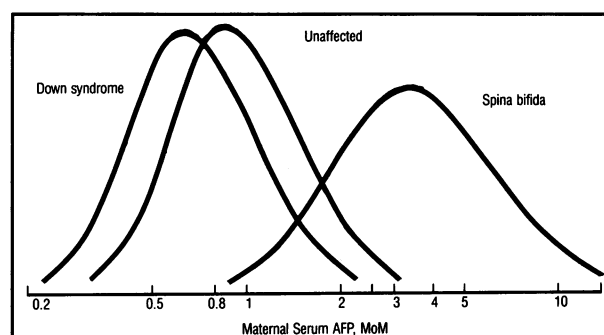
Figure 1.—The graph shows the log gaussian distribution of maternal serum α-fetoprotein (AFP) levels between 16 and 18 weeks' gestation in singleton pregnancies screened for open spina bifida, normal pregnancies, and those with fetuses with Down syndrome. MoM = multiple of the normal median

| TABLE 3.—*Common Causes of False-Positive and -Negative Maternal Serum α-Fetoprotein (AFP) Levels* |
| --- |
| False-positive levels
   Inaccurate gestational dating—patient has a more advanced gestation than estimated
   Multiple gestation
   Race—African Americans physiologically have higher AFP levels than do whites
   Low maternal weight—<41 kg (90 lb)
   Spontaneous fetal-to-maternal bleeding
False-negative levels
   Inaccurate gestational dating—patient has less advanced gestation than estimated
   Maternal insulin-dependent diabetes mellitus
   Obesity |

Maternal serum AFP screening is most accurate when done between 16 and 18 weeks' gestation, but it can be done between 15 and 22 weeks. Screening earlier or later than the optimal gestational age decreases the sensitivity of the test. As with other types of screening, this test should be voluntary and should be done after counseling regarding its limitations and benefits. The patient should understand that a normal maternal AFP result does not ensure a child without an abnormality (including a neural tube defect or fetal chromosome abnormality). Conversely, an abnormal value does not diagnose an abnormality but rather indicates that the patient is at a level of risk that warrants further testing. A protocol for evaluating the pregnancies of women with an elevated maternal AFP level is shown in Figure 2.

Evaluating Elevated α-Fetoprotein Levels

Ultrasonography. Ultrasonography and amniocentesis are used to differentiate the causes of a maternal AFP elevation. Because the underestimation of gestational age is the most common reason for an elevated maternal AFP level, an ultrasonogram to verify gestational age is done first. If the gestational dating used for interpretation is inaccurate, then the multiple of the median is recalculated using the gestational age as determined by the fetal biparietal diameter. If the gestational dating is correct, then a high-resolution ultrasonogram is performed to identify

structural malformations. Several investigators have suggested that ultrasonography alone is an acceptable alternative to amniocentesis for the diagnosis of a neural tube defect, particularly in those women with an AFP level in the range of 2.0 to 3.0 MoM.[6,7] The accuracy of ultrasonography in detecting spina bifida is about 90% and is limited by the location or extent of the lesion, the fetal position, quality of the images, and experience of the sonologist. Thus, a modification of this protocol should be based on these factors.
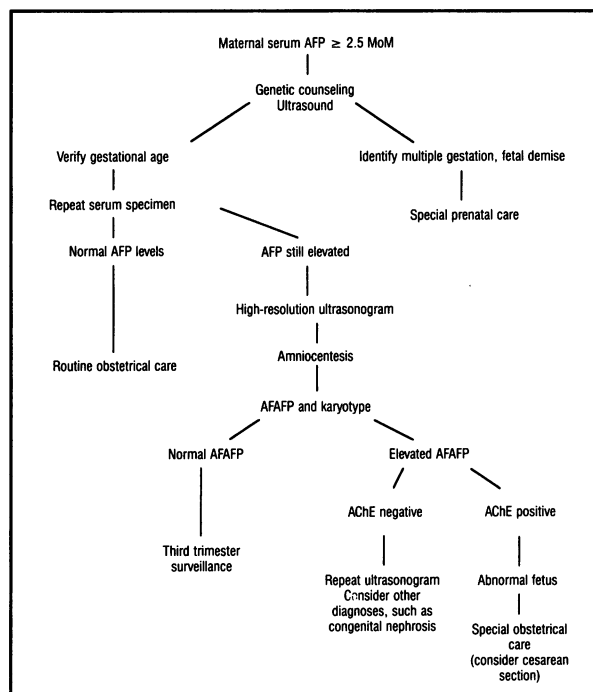
Figure 2.—The suggested protocol can be used for women with elevated maternal serum α-fetoprotein (AFP) levels. AChE = acetylcholinesterase, AFAFP = amniotic fluid α-fetoprotein, MoM = multiple of the median

Amniocentesis. Amniocentesis can be used to differentiate the cause of an elevated maternal AFP value that remains unexplained by ultrasonography. An elevated amniotic fluid AFP level suggests that the fetus has an open defect. A second test for the presence or absence of acetylcholinesterase is used to differentiate a neural tube defect from other fetal defects. Acetylcholinesterase is an enzyme contained in blood cells, muscle, and neural tissue. When the fetus has an open neural tube defect, both the AFP and acetylcholinesterase levels are usually elevated. Elevated amniotic fluid AFP without detectable acetylcholinesterase suggests an open fetal defect other than a neural tube defect. If the patient has an elevated maternal AFP value, normal amniotic fluid AFP level, and normal ultrasonogram, then an open fetal defect is excluded with a high degree of confidence. These women, however, are considered to be at an increased risk for certain obstetrical complications.[8,9] Increased fetal surveillance during the remainder of their pregnancies should be considered.

If a neural tube defect has been identified, a fetal karyotype obtained by amniocentesis and fetal echocardiography are recommended before discussing the prognosis. The morbidity and mortality of such lesions have been extensively addressed by many investigators.[10-12] If the parents elect to continue the pregnancy, serial ultrasonograms are done to detect the development of hydrocephaly and polyhydramnios.

The benefits of cesarean section delivery of infants with spina bifida have recently been reported by Luthy and colleagues.[13] In this retrospective analysis, affected infants born before labor begins had better motor function than those undergoing labor before cesarean section or vaginal delivery. Infants delivered without labor retained greater neurologic function at an average of 3.3 spinal segments above those born after labor regardless of the ultimate type of delivery. There is concern, however, regarding ascertainment bias in this retrospective review of lesions identified antenatally that may have resulted in altered obstetric management.

Preventive Therapy for Neural Tube Defects

Neural tube defects are one of the few malformations for which preventive therapy is available. A variety of temporal and demographic correlations including a higher frequency of these defects in lower socioeconomic classes have contributed to the theory that nutritional deficiencies may be a causative factor. Several studies have examined the use of vitamin supplementation (particularly folic acid) in patients with a history of a neural tube defect during pregnancy.[14-17] In a landmark study recently reported, the recurrence of neural tube defects was prevented by the administration of folic acid.[18] This prospective, randomized, controlled trial demonstrated that women who had had previous pregnancies with neural tube defects had a 72% reduction in their recurrence risk when supplemented with 4 mg per day of folic acid at least four weeks before conception and continuing through the first trimester. Preconceptional folic acid supplementation is currently recommended for preventing the recurrence of neural tube defects in these patients. Because they may recur despite vitamin supplementation, genetic counseling regarding antenatal diagnosis is still recommended for these families.

Screening for Down Syndrome

Maternal Serum α-Fetoprotein

Maternal serum AFP screening for neural tube defects had been successfully applied for about a decade before Merkatz and co-workers reported the association between low maternal AFP levels and fetal aneuploidy.[19] They noted a 25% lower level of maternal AFP in women with fetuses with Down syndrome than in unaffected pregnancies. Several prospective studies verified this association, including a multicenter collaborative study in New England that detected about 25% of cases of fetal Down syndrome (in women younger than 35) with an amniocentesis rate of 3% to 5%.[20]

The median maternal serum AFP value for a woman carrying a fetus with Down syndrome is about 0.8 MoM for normal control pregnancies (see Figure 1). The extensive overlap in the distribution of values between normal pregnancies and those affected with Down syndrome implies that selecting a cutoff level in which women are considered at a higher risk for fetal Down syndrome based on a combination of age and maternal AFP levels involves a greater level of compromise between the detection and the false-positive rate than that for open neural tube defects and the false-positive rate. Most often maternal AFP screening cutoff values are set at such a level that a woman would be considered at an increased risk when the combination of her age at the time of delivery and her AFP level indicates a risk that is equal to or greater than that of a 35-year-old woman. Thus, for women younger than 35, this approach uses a sliding scale of age-specific cutoffs, with a lower cutoff level for younger women and a higher one for older women.[21]

Human Chorionic Gonadotropin

Prenatal laboratories have used maternal serum AFP levels to screen for both fetal Down syndrome and neural tube defects concurrently. Over the past five years, other serum analytes have been reported to enhance the detection rate of fetal Down syndrome. In 1984 Chard and colleagues first suggested that serum human chorionic gonadotropin might be a useful marker in detecting fetal Down syndrome, but they did not offer any supportive clinical data.[22] This was followed by the work of Bogart and associates in which 11 of 17 women carrying fetuses with Down syndrome had hCG levels at 2.5 MoMs or greater[23]; this observation was confirmed by others.[24] Because the median hCG level in affected pregnancies is so elevated in relation to normal controls, hCG is the most effective biochemical marker in Down syndrome screening. In fact, other placental products such as human placental lactogen, progesterone, and the free β-subunit of hCG all have higher levels in Down syndrome pregnancies, suggesting that a hypersecretory or immature placenta may be the source of these abnormalities.

Maternal Serum Unconjugated Estriol

In 1988 Canick and co-workers demonstrated lower second-trimester levels of maternal serum unconjugated estriol in women carrying fetuses with Down syndrome, with a multiple of the median value of 0.79.[25] The synthesis of unconjugated estriol, a steroid hormone, is modulated by the placenta, fetal adrenal glands, and fetal liver. Like AFP, the synthesis of unconjugated estriol is about 25% lower in affected pregnancies.

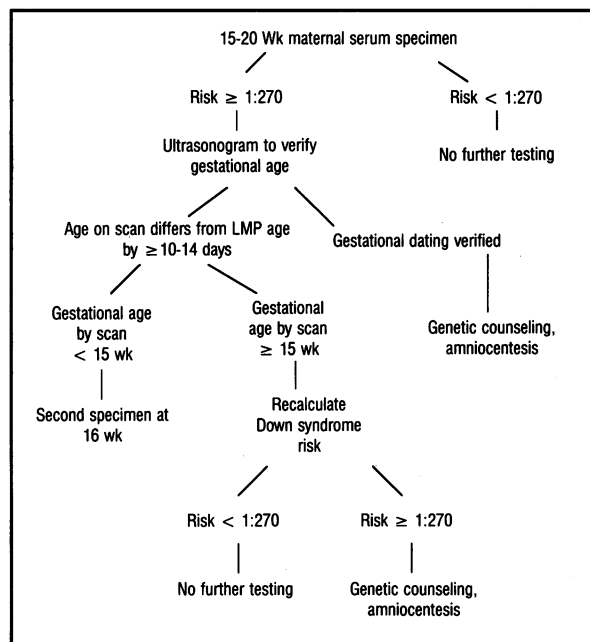### Screening Using All Three Markers

In 1988 Wald and associates compared the serum findings of 77 women carrying fetuses with Down syndrome with 385 matched controls.[26] Gaussian distributions for all three analytes were established. Using a statistical method to combine these distributions with maternal age, individual risks were generated for women

of all ages. Using a risk for fetal Down syndrome of 1:250 (the risk of a 37-year-old woman at term), they identified 67% of cases of fetal Down syndrome. The risk cutoff of a 37-year-old woman was used to maintain the false-positive rate (or amniocentesis rate) at about 5%. This detection rate is significantly better than the detection rate of 20% to 25% with maternal serum AFP alone. Recently three groups have verified this finding prospectively.[27-29] Philips and co-workers evaluated 9,530 women younger than 35 years with these biochemical markers and found 7.2% to initially have a positive test—that is, a risk greater than or equal to that of a 35-year-old woman.[27] After an ultrasound examination to verify gestational age, 4.0% remained positive; 57% of cases of fetal Down syndrome were detected, with a 3.2% false-positive rate. Haddow and associates prospectively screened 25,507 women of all ages (including those older than 35).[28] Again using a term screening cutoff of 1:250, 6.6% of patients were initially positive. Of these patients, only 3.8% remained in the high-risk category after ultrasonogram evaluation, and 58% of cases of fetal Down syndrome were detected. Cheng and colleagues screened 7,718 women of all ages, identifying 6% as positive after an ultrasound examination.[29] In this report, 20 of 22 cases of fetal Down syndrome were identified, yielding a sensitivity of 91% and a specificity of 94%. Therefore, 1 case of fetal Down syndrome was detected for every 23 women offered amniocentesis, and 1 was found for every 17 amniocenteses done. In contrast, if all women of advanced maternal age underwent amniocentesis, 1 case of fetal Down syndrome would be detected for every 140 amniocenteses.

As shown by these prospective series, screening using these three markers is exquisitely sensitive to inaccuracies in gestational dating. If the gestational age is overestimated, then the initial screening test is likely to be positive. For example, consider a woman who is at 16 weeks' gestation, but is recorded as 18 weeks because of poor dating criteria. Because both AFP and unconjugated estriol levels increase during the second trimester and hCG levels decrease during this period, her AFP and unconjugated estriol values will appear low and her hCG value will appear high, a profile consistent with fetal Down syndrome. A two-week discrepancy in gestational dating can change a calculated Down syndrome risk tenfold. To lower the false-positive rate, a woman should have ultrasonographic confirmation of gestational dating by a crown-rump length or biparietal diameter before her test result is interpreted. It is important that femoral lengths or composite gestational dating (biparietal diameter plus femoral length) not be used to determine gestational age because Down syndrome fetuses have shortened long bones on second-trimester ultrasonograms.[30] Depending on the laboratory's protocol, gestational dating is changed when there is a discrepancy between the date of the last menstrual period and ultrasonographic findings of 10 to 14 days.

Currently there are no data to apply this test to multiple gestations or women with insulin-dependent diabetes. There are only limited data for applying correction factors

for weight and race to hCG and unconjugated estriol, although laboratories routinely apply them to the AFP component of the test. A sample protocol is shown in Figure 3.

Figure 3.—A screening protocol using all 3 markers is shown. LMP = last menstrual period

Another advantage of screening with all three markers is the ability to detect trisomy 18. In contrast to the biochemical profile in fetal Down syndrome in which the AFP and unconjugated estriol levels appear low and the hCG values appear elevated, all three markers in trisomy 18 are substantially lower than expected. Therefore, the trisomy 18 risk can be calculated using the same laboratory tests. Using fixed MoM cutoff levels for ten affected patients with an AFP level of 0.75 MoM or less, an unconjugated estriol level of 0.6 MoM or less, and an hCG level of 0.55 MoM or less. Canick and co-workers identified 60% of pregnancies with trisomy 18 that were retrospectively identified with an amniocentesis rate of 0.4%.[31]

Some screening programs omit unconjugated estriol from their protocol and use hCG and AFP testing alone. Clearly, hCG is the most powerful marker for the detection of fetal Down syndrome. Although it only marginally increases the detection rate, there are two advantages of incorporating unconjugated estriol into a screening protocol. This analyte has been shown to decrease the positive rate by about 25%, decreasing the need for amniocentesis and amniocentesis-related losses and costs while maintaining the 60% detection rate. In addition, it is an important component of the trisomy 18 testing protocol. In effect, patients who undergo serum testing for AFP, hCG, and unconjugated estriol levels are screened for three specific disorders: Down syndrome, trisomy 18, and neural tube defects (and other open fetal defects). The incidence of both Down syndrome and trisomy 18 increases with

advancing maternal age,[32] whereas neural tube defects occur on a multifactorial basis.

Maternal Serum Screening for Women Aged 35 and Older

Because women aged 35 or older have a higher empiric risk for fetal aneuploidy, they are routinely offered invasive diagnostic testing for prenatal diagnosis. Because fetal loss is not associated with maternal serum screening, some patients at or older than 35 may consider having a revised estimate of their risk through triple-marker screening before deciding whether to undergo amniocentesis. About 20% of all fetuses with Down syndrome occur in older women; most occur in women younger than 35 years, for whom multiple-marker screening tests are traditionally done. Maternal age plays a pivotal role in formulating the risk for Down syndrome because the risk calculation is heavily weighted by advancing maternal age. Therefore, both the detection rate for fetal Down syndrome in older pregnant women and the amniocentesis (or false-positive) rate will increase sharply in this older age group.[28,29] Although biochemical screening in older women may more accurately determine the risk of fetal Down syndrome than screening the younger age population, it must be stressed to the obstetric patient that this screening test will not detect all cases of fetal Down syndrome and that current standards of obstetric care mandate offering prenatal diagnosis to older women.

Another important limitation of maternal serum screening in the older population is that it does not detect all chromosomal aneuploidies that are increasingly likely to occur with advancing maternal age. Specifically, several sex chromosome anomalies (such as 47,XXY and 47,XXX) would be detected by a prenatal diagnostic procedure, but are not detected by maternal serum screening. The finding of a sex chromosome abnormality raises extremely difficult counseling issues. This is shown by two retrospective series in which 38% and 62% of women informed that they carried a fetus with a sex chromosome aneuploidy elected to terminate the pregnancy.[33,34]

Conclusion

Thus, patients need to understand the benefits and limitations of maternal serum screening before they consent to this voluntary test. Although this protocol does not increase the risk of pregnancy loss in the way invasive diagnostic procedures such as amniocentesis or chorionic villus sampling do, it also does not detect all cases of fetal aneuploidy and does not specifically detect sex chromosome aneuploidies. Biochemical screening, however, has the benefit of a more precise risk assessment for fetal Down syndrome without the risk of fetal loss attributable to invasive prenatal diagnosis.

REFERENCES

1. Brock DJH, Sutcliffe RG: Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. Lancet 1972; 2:197-199

2. Wald NJ, Brock DJH, Bonnar J: Prenatal diagnosis of spina bifida and anencephaly by maternal serum alpha-fetoprotein measurement—A controlled study. Lancet 1974; 1:765-767

3. Report of the UK Collaborative Study on Alpha-Fetoprotein in Relation to Neural Tube Defects: Maternal serum alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Lancet 1977; 2:1323-1332

4. Holmes LB, Driscoll SG, Atkins L: Etiologic heterogeneity of neural-tube defects. N Engl J Med 1976; 294:365-369

5. Ludwin SK, Norman MG: Congenital malformations of the nervous system, In Davis RL, Robertson DM (Eds): Textbook of Neuropathology. Baltimore, Md, Williams & Wilkins, 1985

6. Gray D, Crane J, Johnson C: Ultrasound versus amniocentesis for evaluation of pregnancies at risk for neural tube defects (Abstr). Am J Hum Genet Suppl 1991; 49:1172A

7. Nadel AS, Green JK, Holmes LB, Frigoletto FD Jr, Benacerraf BR: Absence of need for amniocentesis in patients with elevated levels of maternal serum α-fetoprotein and normal ultrasonographic examinations. N Engl J Med 1990; 323:557-561

8. Wald NJ, Cuckle HS, Stirrat GM, et al: Maternal serum alpha-fetoprotein and low birth-weight. Lancet 1977; 2:268-270

9. Burton BK: Outcome of pregnancy in patients with unexplained elevated or low levels of maternal serum α-fetoprotein. Obstet Gynecol 1988; 72:709-713

10. Althouse R, Wald N: Survival and handicap of patients with spina bifida. Arch Dis Child 1980; 55:845-850

11. Bamforth SJ, Baird PA: Spina bifida and hydrocephalus: A population study over a 35-year period. Am J Hum Genet 1989; 44:225-232

12. Hunt GM: Open spina bifida: Outcome for a complete cohort treated unselectively and followed into adulthood. Dev Med Child Neurol 1990; 32:108-118

13. Luthy DA, Wardinsky T, Shurtleff et al: Cesarean section before the onset of labor and subsequent motor function in infants with meningomyelocele diagnosed antenatally. N Engl J Med 1991; 324:662-666

14. Smithells RW, Sheppard S, Schorah CJ: Vitamin deficiencies and neural tube defects. Arch Dis Child 1976; 51:944-950

15. Smithells RW, Sheppard S, Schorah CJ, et al: Apparent prevention of neural tube defects by periconceptional vitamin supplementation. Arch Dis Child 1981; 56:911-918

16. Smithells RW, Nevin NC, Seller MJ, et al: Further experience of vitamin supplementation for prevention of neural tube defect recurrences. Lancet 1983; 1:1027-1031

17. Laurence KM, James N, Miller MH, Tennant GB, Campbell H: Double-blind randomised control trial of folate treatment before conception to prevent recurrence of neural-tube defects. Br Med J (Clin Res) 1981; 282:1509-1511

18. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. Lancet 1991; 338:131-137

19. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE: An association between low maternal serum α-fetoprotein and fetal chromosome abnormalities. Am J Obstet Gynecol 1984; 148:886-894

20. New England Regional Genetics Group Prenatal Collaborative Study on Down Syndrome Screening: Combining maternal serum α-fetoprotein measurements and age to screen for Down syndrome in pregnant women under age 35. Am J Obstet Gynecol 1989; 160:575-581

21. Palomaki GE, Haddow JE: Maternal serum α-fetoprotein, age, and Down syndrome risk. Am J Obstet Gynecol 1987; 156:460-463

22. Chard T, Lowings C, Kitau MJ: Alphafetoprotein and chorionic gonadotropin levels in relation to Down's syndrome (Letter). Lancet 1984; 2:750

23. Bogart MH, Pandian MR, Jones OW: Abnormal maternal serum gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn 1987; 7:623-630

24. Petrocik E, Wassman ER, Kelly JC: Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels. Am J Obstet Gynecol 1989; 161:1168-1173

25. Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ: Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynecol 1988; 95:330-333

26. Wald NJ, Cuckle HS, Densem JW, et al: Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988; 297:883-887 [erratum 1029]

27. Phillips OP, Elias S, Shulman LP, Andersen RN, Morgan CD, Simpson JL: Maternal serum screening for fetal Down syndrome in women less than 35 years of age using alpha-fetoprotein, hCG and unconjugated estriol: A prospective 2 year study. Obstet Gynecol 1992; 80:353-358

28. Haddow JE, Palomaki GE, Knight GJ, et al: Prenatal screening for Down's syndrome with use of maternal serum markers. N Engl J Med 1992; 327:588-593

29. Cheng EY, Luthy DA, Zebelman AM, et al: A prospective evaluation of a second trimester screening test for fetal Down syndrome using maternal serum α-fetoprotein, hCG, and unconjugated estriol. Obstet Gynecol 1993; 81:72-77

30. Lockwood C, Benacerraf B, Krinsky A, et al: A sonographic screening method for Down syndrome. Am J Obstet Gynecol 1987; 157(pt 1):803-808

31. Canick JA, Palomaki GE, Osathanondh R: Prenatal screening for trisomy 18 in the second trimester. Prenat Diagn 1990; 10:546-548

32. Hook EB: Rates of chromosome abnormalities at different maternal ages. Obstet Gynecol 1981; 58:282-285

33. Robinson A, Bender BG, Linden MG: Decisions following the intrauterine diagnosis of sex chromosome aneuploidy. Am J Med Genet 1989; 34:552-554

34. Holmes-Siedle M, Ryynanen M, Lindenbaum RH: Parental decisions regarding termination of pregnancy following prenatal detection of sex chromosome abnormality. Prenat Diagn 1987; 7:239-244